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EDITORIAL

CURRENT JELLY

THE protoplasmic jelly, where life began and in which it continues to have its being, has long been suspected of electric motivation. Indeed it has been stated that all life is electric—whatever that means! A recently published poem, "Short Circuit", defines death as a complete loss of electric current.—It (the poem) ends thus—

> "Which after three score years of going round
> Eventually finds a proper ground—
> Or, if for pyrotechnics I've a flair,
> With all my current gone, I'll get the air".

Certain devices for generating electric current have found specific and valuable uses in medicine. Witness the Roentgen-ray developments with their diversified diagnostic and curative applications.

Then too remember the late—"but not too late" Dr. Abrams, whose electric quackeries, intrigued a following whose name was legion.

Indeed, from the earliest times, electric impulses have been a handy "show-off" for the quick and the quack. The ease with which a quackery progresses is in direct proportion to its mystery and complexity—and, since to this day, so little is known of the reality of things electric, it is only natural that quackery flourishes in the field.

Scribonius Largus, a Roman physician, and a contemporary of Pontius Pilate, is the first recorded user of electric current in therapy. He recommended the Raja topedo fish (said to be able to transmit a considerable charge) in the treatment of headache and rheumatism.

Since his day—and probably before—the laying of hands, pinching, kneading, massaging, osteopathing, chiropractising, mesmerism,

galvanism, hypnotism, heliotherapy, ray-therapy—these and many other forms of electric treatment have had their way and their day—not only with the quack and the empiricist—but to a measure—and perhaps to an inadequate measure—with the so-called legitimate practitioners of medicine.

That this electric field is important to those who care for the public's health is becoming more evident every day. There is quoted herewith a survey of certain advances in concepts and in devices that are of very recent origin. The quotations is from that splendid little bulletin (Industrial Bulletin, Arthur D. Little, Inc., Cambridge, Mass. —June, 1936—No. 113).

"It has long been surmised that electricity has much to do with the human body, particularly with the nerves and the messages they convey. This impression perhaps started in 1791, when Galvani announced the result of his extraordinary experiments with frogs' legs, wherein he observed that some strange impetus associated with metals in contact could cause brisk contractions of the thigh muscles. Galvani unwittingly had made a battery of pieces of copper and zinc, the salt and moisture of the frog's leg furnishing the electrolyte, and with it secured the same type of response that was obtained when he used the discharges of a static electric machine. In addition to this work by Galvani, there came wonderful tales of electric eels in South American tropical rivers, the shock of which could kill a horse.

Until quite recently, all attempts to detect electrical currents in the animal body gave either negative or very vague results. Now, however, it is possible, by using electrical amplification of the general type employed in radio sets, to increase enormously the minute potentials or currents under investigation, to make them operate suitable meters, loud speakers or even recording machines. The means is thus at hand for intensive study of the electrical quantities associated with nerves and muscles, and perhaps even with thought. Great care is needed, however, in judging the reliability of some of the more startling claims.

The uncanny skill of the electrocardiograph in detecting intimate details of the heart and its history and performance is becoming well known. Various types of emotion detectors are being experimented upon, with the possibility that they may be of assistance in criminal cases. By measurement of body 'impedance' to the flow of high-frequency current it appears that thyroid disturbances and perhaps

other diseased conditions may be diagnosed. Certain workers on dementia præcox are experimenting with electrical apparatus for diagnostic purposes.

It appears that whenever we see, hear, taste, smell or feel, the sense organs involved transform their findings into electrical 'action currents' which travel along the nerves to the brain. It is everyday technique in certain physiological laboratories, for example, to tap the auditory nerve, and, by amplification of the feeble action currents, to obtain strong electrical impulses that can operate loud speakers. Much has been learned about the sense of hearing by this electrical study. We now know, for instance, that up to moderate frequfiencies, at least, cats' ears respond as do our own.

Other workers have shown that the taste buds disposed along the sides of a fish give electrical impulses when stimulated by sour or bitter things (though not by sweet), and that the optic nerve of a frog's eye shows analogous impulses when light is allowed to fall on the retina. In both of these cases, the impulses appear to be alternating currents with frequencies characteristic of the intensity of the 'sensation,' if it may be so-called when the sense organis separated from the brain.

Workers connected with medical schools believe that 'brain waves' may yield much information about epilepsy, for it is possible to detect by their means the stages of onset of the great and small seizures of this disease, and locate the affected brain centers, so that removal may be possible. These waves are the electrical tremors that can be detected through the skin at the top of the head of a relaxed person, whether awake or asleep. Electrical study of the action of drugs on the body is just beginning to arouse interest.

The interpretation of what has already been discovered about nerve impulses will take much care and effort, for there are still many alternative explanations of what the impulses may mean. All that may be safely said is that here is a powerful tool for the exploration of nerve and brain, and perhaps of the body in general, also of such phenomena as consciousness and thought, and much may be expected of it in the future. So far, the applications of electricity to the body have been largely diagnostic. It is fascinating to imagine that they may yet be found potent for relief, for anæsthesia, or even for the cure of certain diseases."

IVOR GRIFFITH.

ORIGINAL ARTICLES

THE HISTORY AND ROMANCE OF DIGITALIS†

By John E. Kramer, Ph. G.

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AFFAIRS of the heart, of the more pleasant variety, have besieged mankind, and womankind, for eons and eons. Adam and Eve had one, Anthony and Cleopatra had one. And, if you were diligent in your newspaper reading at the time, you will remember the more recent famous, or infamous, affair of the hearts of John Barrymore and Elaine Barrie. I've had one, and I hope each of you here tonight has had one.

John E. Kramer, Ph. G.

But in one year, 1934, 6218 people in Philadelphia had affairs of the heart that were not so pleasant. In fact, they died from them. Figures from the coroner's office indicate, moreover, that during the first nine months of 1935, 4473 other Philadelphians died from diseases of the heart, a number greater than from any other single cause, and twice as great as cancer, its nearest rival. Other statistics show that heart disease fatalities have increased more than 50 per cent. in the past fifteen years, while the general death rate has dropped. In addition, many sufferers from cardiac troubles are lingering a living death, incapacitated and altogether useless to society.

Now, are you all depressed enough? Are you feeling your pulses and wondering if you are to be the next victims? Well, cheer up, for the rest of the discourse will concern itself with that most popular, most common, most important of all cardiac drugs, or, as I please to call it, Digitalis, heart's delight.

From an obscure, little-thought-of herb among many others in a haphazard mess prepared by a garrulous old woman in Shropshire, England, Digitalis has grown, in a period of 170 years, to be the universal drug prescribed in most cases of heart disorders. Although a native of the meadows and pastures of Europe, most of the

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Digitalis used in this country is cultivated on drug farms, notably in the West, and it grows wild in the States of Washington, Oregon and New York. In addition, anywhere from 15,000 to 70,000 pounds of Digitalis leaves are annually imported into the United States for the express purpose of manufacturing standard, and standardized, pharmaceutical preparations.

It might be well, at this time, to give some description of our subject, so that we all know just what we are talking about.

The Digitalis of the Pharmacopœia of the United States, the official book of pharmaceutical standards in this country, is described as the carefully dried leaves of Digitalis purpurea Linné, a biennial herb. During its first year's growth, the herb consists of a group of leaves, ovate-oblong in shape, and seeming to spring from a central point and extending in all directions, somewhat like the foundational leaves of a bouquet. The second year, however, a thick stem shoots up, sometimes to a height of a yard or so, and the leaves extend outward from this vertical source. A beautiful flower, for which the plant is sometimes especially cultivated, appears in the second year.

When cultivated, the plants are propagated in February or March by mixing the seeds with sand and placing them in hothouses. In the spring they are transplanted to large well-drained fields. Leaves of both years' growths are collected, and dried, either spontaneously in the shade or in ovens. In drying they lose about 75 per cent. of their weight. Powdered dried leaves are dark green in color.

The plant is variously known as Foxglove, Folk's Glove and Ladies' Glove, Dead Men's Bells and Purple Foxglove, all outcroppings of its eary history, when it was merely an herb that was avoided as poison or blunderingly used as a cure-all.

Many drugs in use today were old and familiar to the ancients. Digitalis is not among that select group, its first mention being in a Saxon Herbarium in the year 1000 A. D., just about the time of the first Crusade, when the brave and brawny holy men of Europe left their firesides in great indignation to make what was then known of the world safer for Christianity. The drug was listed in this book as "Foxes Glofe."

The Welsh "Physicians of Myddfai" of the Thirteenth Century were familiar with the good results of using this mysterious herb in decoctions and ointments.

It was not until 1542, however, that the present name was originated. Dr. Leonard Fuchs, who was more of a botanist than a physician, was writing and editing his famous "De Historia Stirpium" and was faced with the problem of classifying "Foxes Glofe," known in the German as "Fingerhuth." So he translated it into Latin, modified it slightly, and entered the drug in his good book as Digitalis purpurea.

As is very often the case, some historians do not agree with this story of origination of name. They give the credit to Hieronymus Bock, whose nom de plume was Tragus, and who mentioned Digitalis purpurea in a book he published in 1552.

From this time until 1775, when we find records of the first intelligent study of Digitalis, the herb had its ups and downs, and was kicked about like a stepchild by the medical profession. In 1597 it was recommended as good to "cut and consume flegme and humours, and to scour and clean the breast." In 1600 a decoction of it was thought good for gargling, to correct inflammation and fever, while the leaves cured bowel problems, and in 1618 it was vouched for as a treatment for epilepsy, scrofulus and ulcers. In 1706 it was decided that Digitalis was an anti-asthma remedy and an expectorant, confirming the opinions of the learned gentlemen of 1597.

But from 1630 to 1719, no less than six authorities said that Digitalis had no tonic values, and in 1727 one author deemed the drug too acrid and poisonous for internal use.

In 1740 still another so-called authority claimed it to be "as efficacious as any drugs the Indies produce," and in 1761 we find a compromising statement to the effect that Digtalis was too violent for internal use but was excellent for sores, etc., etc., etc.

It is to be noted that the drug appeared in the London Pharmacopœia of 1650 and 1720, was an outcast in 1775, but was taken back into the fold in 1788.

And it was exactly 161 years ago, in 1775, just one year before our country's Declaration of Independence, that a certain English physician treated a case of heart trouble in such a manner as to prove to be a revolution in the world of medicine and a sort of Declaration of Independence for heart sufferers.

Let us tell the story in the words of Dr. Edward Podolsky, a practicing physician of Brooklyn, and the author of many articles

on current medical topics, who wrote recently in the American Journal of Pharmacy concerning the early history of Digitalis.

"Dr. Cawley, the Dean of Brazen Nose College, Oxford, was sick. He was lying in bed, breathing hard and he felt tired in every fiber of his being. Even in bed he felt like a man who had been running a long, hard race. His heart was beating fast and furiously. His legs had grown to almost twice their natural size and the skin on them was tense and glazed; here and there was a small crack in the skin through which a drop of water exuded now and then. The Dean's belly was big, big with stagnated water accumulated from a sluggishly moving blood stream. It did not require much more than a glance to see that Dr. Cawley was suffering from a failing heart.

"The Oxford Dean had many friends among the doctors. They came to his bedside and shook their heads gravely. They bled and sweated him. They purged him with calomel and jalap. They forced squills upon him. They scarified his skin to let the water out. But in spite of all their efforts Dr. Cawley lay in bed, breathing hard, alarmingly hard and filling up with water.

"In the hospital at Birmingham one of the junior members of the staff, young Dr. William Withering was gaining some sort of reputation with a new medicine for the dropsy. Dr. Withering had been a physician for not more than eleven years. At times he was more interested in plants than in human beings. He gathered the plants of the countryside on his off hours; he described and classified them according to characteristics. He carefully collected his notes for some future day when he intended to write a book based on them.

"When Dr. Withering had been called in to treat the Dean of Brazen Nose College he was firmly convinced that foxglove was the one medicine which would help him. He prescribed a solution of the drug which he himself had carefully prepared. With the passing of the hours the medicine began to work wonders. The Dean's face became natural in color; his heart began to beat slower and in more ordered rhythm. The water left his legs and belly. To the end of his days Dr. Withering prized a letter which he had received from Dr. Cawley's brother who insisted that Dr. Withering with his foxglove had saved the Dean's life on that occasion and had prolonged it for a year."

This now-famous physician had come into first contact with foxglove through hearing of an old woman of Shropshire, in Wales, who had been curing dropsy after the recognized medical men had miserably failed. In order to gain the secret of her success, Dr. Withering cajoled her into revealing the identity of the score of herbs she used in her very efficacious decoction. Then, after much experimentation, he determined that foxglove was the only valuable herb in the lot. He also recognized this plant as one mentioned by the very old Welsh medical books as having worth in the treatment of cardiac disturbances.

Other rural English and Welsh communities had long used foxglove in cases of dropsy, and why not, for it grew as a weed all through the countryside, was used as a floral decoration by the elders and by the children as bee traps, and was bound to be included in any mess of pottage collected for medicinal or culinary use.

With this fact determined to his own satisfaction, Dr. Withering started a long series of experiments on a great number of unwitting, but who he was sure would be willing, human guinea pigs. He helped many hearts, disabled for numerous reasons, and his almost unbelievable success began to attract attention. His colleagues commenced to use the drug, and, meeting with a similar success, proclaimed its virtues far and wide, and acclaimed Dr. Withering a real hero, if ever doctors are acclaimed as heroes for saving lives in this manner.

In passing, let us reflect how strange this world is in hailing as brave and true those who lead or direct groups of men with the express aim of wiping out other groups of men. Such "heroes" come and such "heroes" go, but, thank goodness, medicine and its allies go on forever in the never-ending battle against pain, sickness and disease.

As a direct result of his extensive experimentation, Dr. Withering, in 1785, wrote a book entitled, "An Account of the Introduction of Foxglove Into Modern Medicine." This work was, and still is, a classic of medical literature.

Please bear in mind that this work was done in the ten-year period between 1775 and 1785, and, although it met with recognition, as mentioned, there were no great medical associations and journals to which to report, no cables to carry the news quickly, no radio to dramatize the findings for the whole world of listeners. Further-

more, there was still a great amount of ignorance, prejudice and innate backwardness to be overcome.

Therefore, many more medical men experimented and propagated the virtues of Digitalis before it became universal. Such names as Duncan, Stokes, the German Smideberg, Eggleston, Gold, Otto, Hatcher and many others are irremovably connected with the progress of Digitalis.

In searching and researching through the dusty files of the literature of dim yesterdays, your lecturer came upon a book, the very title of which would seem to put its author in that group of men mentioned a second ago. It was in 1807, in London, that the good physician, Dr. William Hamilton, had printed (take a deep breath) "Observations on the Preparation, Utility and Administration of the Digitalis Purpurea, or Foxglove, in Dropsy of the Chest, Consumption, Hemorrhage, Scarlet Fever, Measles, etc., Including a Sketch of the Medical History of This Plant, and an Account of the Opinions of Those Authors who have written upon it during the last thirty years." This book is interesting, and is illustrated by actual case histories showing evident favorable use of Digitalis in instances of all the illnesses mentioned in the title.

Enough, now, for the history of Digitalis. And so that we might delve into the modern concepts of its constitution, its uses and its methods of administration, it seems almost necessary to first make a short resume of the recipient of all this attention, the heart.

Although this great pumping station of the body's blood is not as plain and as evident as the nose on your face, nearly everybody knows most of the standard facts about the heart. Ever since we were grammar school children and saluted the flag each day, we knew that the heart is located in the upper chest, slightly to the left of center. We are also more or less familiar with the following general facts, stated in brief:

It is about the size of a clenched fist.

It weighs about nine ounces.

It starts to beat before the birth of its human host.

It beats about seventy to eighty times a minute in the fully grown person.

It is a hollow muscle.

It is divided into two great parts, and each of these is in turn divided into two parts. These parts are known as ventricles and auricles.

It contracts, forcing the blood, containing oxygen, into the arteries, in an act known as systole.

It then relaxes, and fills itself up again in an act known as diastole.

It forces the blood through the body at the rate of about once a minute.

It rests between each beat, awaiting the stimulus for the next contraction.

It can be kept beating artificially even though not in its proper place in the body.

It beats regularly, without conscious stimulation by its owner, through a system of its own.

It normally pumps seven and one-half tons of blood each day.

In a person of forty it maintains the blood at a pressure of about 130 mm.

The blood, itself, forced to every part of the body by the heart, is still another story. Strange as it may seem, the circulation of the blood was not discovered until just a little over 300 years ago. It was in 1628 that Dr. John Harvey, private physician to King Charles I, took advantage of his nearness to royalty to publish his startling findings after a series of experiments. The simplicity of the thing makes it the more amazing to us today.

Here is the experiment, in Harvey's own words:

"Let an arm be tied up above the elbow as if for blood-letting (phlebotomy) (A, A, Fig. 1). At intervals in the course of the veins, especially in laboring people and those whose veins are large, certain knots or elevations (B, C, D, E, F) will be perceived and this not only at the places where a branch is received (E, F) but also where none enters (C, D); these knots or risings are all formed by valves, which thus show themselves externally. . . . Apply the thumb or finger over a vein in the situation of one of the valves in such a way as to compress, and prevent any blood from passing upwards from the hand; then, with a finger of the other, streak the blood in the vein upwards till it has passed the next valve above, the vessel now remains empty; the finger being removed for an instant, the vein is immediately filled from below; apply the finger again and having in the same manner streaked the blood upwards, again remove the finger below, and again the vessel becomes distended as before; and this repeat, say a thousand times, in a short space of time. And

now compute the quantity of blood which you have thus pressed up beyond the valve, and then multiplying the assumed quantity by one thousand, and you will find that so much blood has passed through a certain portion of the vessel; and I do now believe that you will find yourself convinced of the circulation of the blood and its rapid movement."

The publication of this work practically revolutionized the practice of medicine at that time.

Having, now, more or less of a clear mental picture of the drug Digitalis on one hand and the heart and its actions on the other, let's see what happens when they get together, for, unlike East and the West, the twain meet very often. Let us remember, however, that the enforced meeting of these two great evidences of nature's handiwork usually occurs only when the heart is weak and the Digitalis willing.

Upon administration of the drug in any one of its many prepared forms, the blood pumper immediately is stimulated, and has a tendency to more rapid action if it had previously been running behind schedule, or, if it had been racing, Digitalis will slow it down more nearly to normal. In other words, Digitalis is the great stabilizer for those hearts beating in three-fourths time when they should be marking common, or four-fourths tempo.

All of this equalization is evidenced by a corresponding regulation of what was probably an irregular pulse, which may be likened to the meter on your electric line, indicating the workings of the central station and the supply to the remote parts of the system.

This relief to needy hearts is brought about in two distinct actions. Although Digitalis, in moderate doses, does not alter the time of systole, it does prolong the diastolic period, and this being the time of rest and recuperation after the preceding systolic push, the benefit is self-evident.

Digitalis also increases the force of the push sending the blood through the arteries, and although this would seem to give the organ more work to do, it really is doing good because the blood being pumped is of better grade.

In small and moderate quantities, the drug acts as a diuretic, this being one of the actions so helpful to Dr. Withering in his treatment of the dear old Dean of Brazen Nose College.

It might be assumed, with some degree of fairness, that small or moderate doses of Digitalis are beneficial, twice the average dose would be twice as helpful, and that three times the average dose would be thrice as efficient. Such is most emphatically not the case, however, for with doses that are too large the heart beats more rapidly than ever and the contractions become more irregular than ever and more forceful, too, at first, forcing out the heart's own nutrient blood supply. Following this the heart beats progressively faster, but with less force, and, with toxic doses of the drug, runs away entirely and finally stops exhausted, figuratively and actually dropping dead, and its owner following suit.

Observed symptoms of Digitalis poisoning follow the foregoing phenomena very faithfully. First, the victim feels faint and complains of headache, giddiness and nausea, this latter symptom being evidenced very often by many persons who are sickened after taking even a moderate dose, as the drug has a direct stimulating action upon the vomiting center. Abdominal pain and purging are next in order for the unfortunate who is suffering from an overdose. His pulse is very irregular, he is pale and weak and has a subnormal body temperature. Although lethargic, he is fully conscious, until there occur a series of convulsions, followed by coma and merciful death from heart paralysis.

A person suffering from too much Digitalis is immediately placed in a horizontal position and kept there, even for several days after treatment (if it is successful) so as not to exert the heart one whit more than is absolutely necessary. The stomach is cleansed thoroughly with tannic or gallic acid or whatever emetic is handy, and a saline cathartic is administered. Atropine or nitroglycerin is given hypodermically as an antidote, as are other stimulants of all kinds, and, if near coma sets in, artificial respiration is resorted to.

Cases of poisoning, the symptoms of which were just described, were probably well known to the pioneers in the use of Digitalis. Very likely their greatest worry was that of the uncertainty of the strength of the drug they administered. Hit and miss methods must certainly have been the order of the day, but the untimely ends of some of these unfortunate patients made the physicians cast about for the answers to four great questions. What is it in the Digitalis plant that is responsible for its action? What is the best method for administration? What is the proper dose? How can one be sure of the strength of the prepared drug?

In order to answer these very perplexing questions Digitalis has, since the days of Dr. Withering and his followers, been the subject of as much, if not more applied research than any other drug. Even today all of the questions have not been fully answered, at least to the satisfaction of some.

It is believed, however, that the action of the plant is due to the presence of a number of glucosides, chemical substances which can be decomposed to yield glucose. Chief among these glucosides are digitoxin, gitalin, digitonin, digitalin and bigitalin.

There are a number of tried and true methods of administration. First is the powdered drug, which is the leaf after it has been dried at a temperature not above 60°C. and reduced to a fine powder, and which must contain less than 5 per cent. of moisture. This product is official in the Pharmacopœia of the United States.

The tincture, also official in the eleventh decennial revision of the U. S. P., is prepared by dampening a certain quantity of finely powdered Digitalis with a portion of a mixture of four volumes of alcohol and one volume of distilled water. The moistened drug is then transferred to a percolator, shaken down, allowed to stand for a short time and then packed firmly. More of the alcohol water mixture is added to saturate the powder and the whole thing allowed to stand for a day. Then the outlet at the bottom of the percolator is opened, the liquid allowed to drain, and more mixture percolated through, until a certain quantity is obtained. The finished product is then assayed and made to conform to a definite standard of potency.

An infusion of Digitalis is official in the other book of pharmaceutical standards, the National Formulary, now in its sixth decennial edition. Boiling distilled water is poured over the powdered drug and allowed to stand for an hour. It is then filtered and alcohol, to which spirit of cinnamon has been added, is mixed with the filtrate.

It should be noted by those who are interested that these preparations are incompatible with tannin and other vegetable astringents and with substances that precipitate the chemical group of alkaloids. By incompatible we mean that the two cannot exist in the same preparation and still retain their own identities, and, in being incompatible, they lose their effect. They cannot, therefore, be prescribed together, and it will be remembered that tannic acid and gallic acid were listed as specific antidotes. To better explain this matter of incompatibilities, it might simply be said that preachers do not patronize night clubs, pussy cats do not hobnob with mice, and little

Johnny is not a welcome visitor to the parlor when big sister is entertaining her boy friend. They are all incompatibles.

Digitalis is also prepared in tablet form, in capsules, in pills, and in sealed glass ampuls.

Various pharmaceutical manufacturers have developed special Digitalis preparations. For instance, one company markets a fat-free tincture prepared from fat-free drug and supposed to be less disturbing to the stomach.

The average dose of standardized powdered U. S. P. Digitalis leaves is one and one-half grains. Ten cubic centimeters of the tincture is equal to one gram of the leaves, making the average dose fifteen minims. One hundred cubic centimeters of the infusion is equal to 1.5 grams of Digitalis leaves, making the dose of this preparation ninety minims.

Absolute need of certainty of the accuracy of dose of this potent drug has led one manufacturer to dispense "dropsule bottles," glass vials equipped with dropper gadgets permitting accurate, regulated flow of liquid insuring constant dosage in each drop. These bottles also prevent the liquid from being exposed to air contamination and evaporation.

Comes now the fourth question—a very important one. How can one be sure of the strength of the prepared drug? No matter how accurate we are in administering the preparation, if we are not absolutely sure that a certain amount of it contains a certain amount of the active principle of the drug, our pains are wasted.

It was obvious to medical and pharmaceutical research workers that a standard of strength must be established and that in the establishment of this standard some animal, or animals, must be used as live testing grounds. This in view of the fact that chemical methods of assay are not yet practicable. So, after much meditation, collaboration and finally corroboration, the very serviceable frog was chosen. Guinea pigs, dogs, cats, rabbits, and other forms of life, even goldfish, have been proposed and thoroughly and exhaustively worked upon, but faithful Rana pipiens Schreber, known to most of us as the frog, was elected.

A recent suggestion by Dr. Arno Viehoever, research professor in biology at the Philadelphia College of Pharmacy and Science, brings forth the lowly daphnia, erstwhile fish food, as a test animal. This minute creature is transparent, affording view of its heart, digestive system and other organs through the microscope. Drug

actions on these organs can plainly be noted, and comparison of various strengths of drugs can be made. Further experimentation is being made in this fascinating and fantastic field.

The present accepted method of assay is, in brief, as follows: An accurate standard preparation of Digitalis is obtained from the U. S. P. central office, and one cubic centimeter of this preparation represents one U. S. P. Digitalis unit, and is then used as the basis of comparison for the preparations to be assayed.

Healthy frogs, two to three years old, weighing between fifteen and thirty-five grams, and having been kept under prescribed conditions, are accurately weighed and divided into two groups. Into one group graduated quantities of previously diluted standard preparation are injected, these quantities calculated to be the equivalent of progressive amounts of drug per gram of body weight of the animals. Similarly calculated quantities of the preparation of unknown potency are injected into the second group of frogs.

Exactly fifty-eight minutes later they are rendered unconscious, are not susceptible to pain, and their hearts exposed. At exactly one hour they are examined and the groups compared. If the conditions of the hearts of the unknown group are not similar to the control group, other frogs are injected with larger or smaller doses of the unknown, according to the need. When uniform results are obtained, the potency of the unknown is calculated from the doses given to obtain these uniform results, and the unknown is then modified to meet the set standards of strength. This is known as the "one-hour frog method," and is the only official method of assay.

It must have been noticeable to you listeners this evening that care, accuracy and exactitude follow Digitalis from the heart of its seed to the heart of its human recipient. Before planting, the seeds are most expertly selected, and constant and unremitting attention is given them during cultivation and growth. Inferior plants are weeded out from the good, and only leaves of proper age, size and appearance are chosen to be dried to specific moisture content for controlled storage, subsequent supervised scientific assay and preparation for administration.

The thoroughly capable doctor examines the heart of his ailing patient, prescribes Digitalis if he thinks it is needed, and the well-trained pharmacist fills the prescription accurately.

All of this, ladies and gentlemen, to assist us in obeying the very wise admonition of the Bible to "keep thy heart with all thy diligence, for out of it are the issues of life." (Proverbs 4:23.)

THE MICROSCOPY OF CERTAIN OF THE POWDERED DESICCATED ENDOCRINE GLANDS*

By Paul Alvin Mattis

Introduction

THE use of the various organs of the body as remedial agents must have arisen early in the history of man. We know that primitive peoples held to the naive belief that the consumption of the various organs of their fellowmen was followed by an increase in some particular power or powers. Thus the savage ate the heart of his enemy to enhance his own courage.

In Hippocrates time, physicians thought that the various animal organs were of remedial value. In fact, Dioscorides in his Greek Herbal, compiled during the first century A. D., lists various animal organs to be used in the treatment of manifold diseases. For example, he lists the brain of the land hare as being useful in nervous conditions; the lungs of the fox he considered of value in respiratory disorders; the liver of a mad dog in the treatment of hydrophobia; rennet of the horse or hare for ailments of the stomach and intestines. Pliny the Elder also recommended the use of the testicles of the stag and the donkey as aphrodisiacs.

The conception of organotherapy was not original with the Greeks, but according to Hofler (1908) arose with the doctrine of signatures (similia similibus curantur). Hofler found that of the many animal parts used as remedial agents the majority were employed in a very indiscriminate manner, depending more on mythological connections than on sound therapy.

Though Berthold (1849) was undoubtedly the first to demonstrate internal secretions by experimental means, it remained for Claude Bernard (1856) and Brown-Sequard (1889) to found the doctrine of internal secretion as we know it today. Claude Bernard's work did not receive the attention accorded the pronouncements of Brown-Sequard, who gave impetus to investigations which have reached tremendous proportions.

The great strides made in the last decade in our knowledge of the physiology of the endocrine glands has resulted in the appearance

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on the market of numerous glandular products intended for therapeutic use. Among this assemblage are the powdered desiccated gland substances. Few indeed are the portions of the animal body which have not been dried and powdered for application in the art of healing.

The increased use of these substances has made it necessary to find methods of standardization. In some instances, standards are now worked out defining the potency of the products. These standardization methods are in most cases physiological in nature. Though physiological tests may be an indication of the purity of the product they are not sufficient in the case of the powdered glandular products, because by their nature it would be an easy task to adulterate the powders.

With this in mind, the author set out to identify the various histological elements found in certain of the powdered glandular products. It was hoped that the histological elements in these powders normally present and recognizable by microscopic structure might be listed, with a description of their appearance. The powders selected for this study were the following, powdered desiccated whole ovary, powdered desiccated corpus luteum, powdered desiccated ovarian residue, powdered desiccated thyroid, and powdered desiccated whole pituitary.

In working on this problem, many methods were of necessity used in examining the powders. From the constant employment of and comparison of the methods, certain procedures have been found advantageous in routine examinations. Although not a part of the problem, attempts were made to find simple color reactions for each of the powders examined.

An extensive survey of the literature disclosed but few articles concerned with this problem. Professor Youngken (1933) published the first of a series of three papers on the microscopy of the glandular products. Certain of the French workers have made studies on the powdered glandular products but these have been for the most part concerned with the chemistry. Javillier, et al. (1925) published a paper on a new method of assay of the organotherapeutic powders. In the same year, the report of the XIV Sub-Committee of the Codex (1925)—dealing with organotherapeutic products—published a report on the description of these same products. Again their concern was with standard chemical methods of assay rather than any micro-

scopical description. Mention was briefly made here, however, of the possibility of detecting adulterations by microscopic means.

The author has just received the recently published manual for pharmacognostic research edited by R. Wasicky (1936) in which a section on the animal drugs has been included. The microscopy of the powdered animal drugs is here listed in some detail together with certain staining reactions.

Methods

The powders used in this study were secured through the kindness of Armour and Company. The reagents used in making the various solutions were either Merck's Blue Label Chemicals or Baker's Analyzed Chemicals (C. P. Baker's Analyzed). Only the purest stains available from Grübler or Harleco were used in this work.

Early in the study use was made of prepared slides of the glands studied. These had been stained by various methods—namely, with Delafield's hematoxylin and eosin, Heidenhain's hematoxylin and orange G, and Mallory's triple connective tissue stain—and permanently mounted in balsam. These sections were compared with descriptions in several histological texts. Upon the examination of the powdered products it was at once evident that their microscopic examination would give a far different appearance from text-book pictures and slides prepared from microtome sections of the glands.

The methods the author used in preparing the powders for microscopic examination may be divided into two types; those in which the desire was to obtain permanent preparations for prolonged study and those intended to give relatively temporary mounts.

In order to secure permanent mounts of the powders it was decided that balsam mounts would be most satisfactory. This was easily accomplished with the dry unstained powders as it was only necessary to wash in 95 per cent. alcohol, then in xylol and mount directly in balsam. In order to make permanent balsam mounts after using aqueous stains dehydration was necessary. Because of the small size of the individual particles of the powders, the transference from one solution to another without undue loss of material was somewhat of a task. Several methods were tried, some of which were discarded as having no value. The following methods of the many tried were found to have definite value in making permanent preparations and reduced loss to a minimum.

All glassware and materials used in working with the powders were kept scrupulously clean, because of the danger of introducing adulterations. All slides and cover-glasses were washed in cleaning solution, rinsed in water and stored in acid alcohol. Before use, they were dried with a new linen cloth which had been washed to free it of lint. The centrifuge tubes and test tubes were carefully washed with a brush, soaked in cleaning solution, and rinsed with distilled water at least three times before using.

Watch glass method. The powder to be stained was placed in a Syracuse watch glass or embryological staining dish. The latter was found useful because of the rounded bottom. The stain properly diluted was introduced and allowed to act for a sufficient length of time. It was possible with certain stains, where the color was not too deep, to place the watch glass under a microscope and observe the depth of the stain at varying intervals. The stain was removed by means of a medicine dropper and water or alcohol added to wash out the excess stain. After washing, alcohol was added in gradually increasing strengths until 95 per cent. alcohol was reached (e. g. 50%, 75%, 83%, 95%). It was found advisable to clear in clove oil or aniline oil after dehydration in alcohol, after which the oil was washed from the powders with xylol. The powders were then ready for mounting in balsam. It must be noted that this method was modified to suit the staining technique used, as some stains would be withdrawn by the use of alcohol. The elimination of certain percentages of alcohol in dehydration was found to make little difference in the final appearance of the powder.

It was also found advisable to use separate pipettes labelled for each powder since in removing the stains or alcohols it was almost impossible to prevent getting some of the powder in the barrel of the pipette. To use this pipette for other powders was to introduce an error or adulteration.

The author has found this method to be of value in staining and preparing powders for examination by temporary mounts. A fair bulk of the powder could be stained in the dish and many mounts made from this one preparation.

Filter Method. Another method sometimes of value utilized a sintered glass filter. The powder was stained in a watch glass or embryological dish and the powder and stain transferred to the filter when sufficiently stained. By placing the filter in a filter flask and

maintaining a gentle suction, it was possible to wash rapidly with water and ascending grades of alcohol until finally the powder was brought into xylol. Since xylol passed through the filter very rapidly, suction was removed at this stage and the powders transferred with xylol to an embryological dish before mounting. This method, though of use in effecting rather rapid dehydration, presented the problem of loss of some of the powder since particles remained caught in the interstices of the filter. These had to be very carefully removed and the filter washed thoroughly with cleaning solution before being used for another powder.

Ordinary paper filters were tried, but in washing the powder from the paper one sometimes found bits of this material in the mount.

Centrifuge method. This method consisted essentially of staining the powders in the diluted stain in a centrifuge tube and then centrifuging. The powder remained packed in the bottom of the tube and the stain was easily poured from the tube. Several washings with water and treatment (centrifuging) with ascending grades of alcohol followed. When in xylol and clear, the powder was easily recovered from the bottom of the centrifuge tube by means of a long glass tube or pipette.

The paraffin method. This consisted essentially of the dehydration of the powder, clearing in oil and embedding in paraffin. The method used may be found by referring to any of the standard text books on histological procedure. Certain precautions must be followed in using this method. It was observed that the powder cleared rapidly in oil and that the time in the paraffin bath should be as brief as possible in order to prevent hardening of the powders. Several methods of embedding the powders were tried, none of them yielding consistently favorable results. One device used a cylinder (1 millimeter in diameter) of hard, smooth-surfaced paper made by rolling it over a test tube and sealing by linen hinges. A cork was used to plug one end of the cylinder and into the *warmed* cylinder was introduced the powder in melted paraffin. Instant immersion in ice-water caused rapid hardening, and on removal of the paper, the paraffin core was mounted on a wooden block and trimmed for sectioning on a microtome. This procedure has not been uniformly successful. Many problems remain to be solved before the method may be said to be consistently successful. Two factors which caused poor results

with this method were the lack of uniform distribution of the powders in the paraffin cylinder and the hardness of the powders resulting in crumbling of the sections. When successful sections were made by this method and subsequently stained, the appearance of the tissue was equal to any of the other preparations and far superior to many mounts.

Smear method. The basis of this procedure was the fixing of the powders to a glass slide in a more or less thin film, so that they could be stained and run through alcohols without the danger of loss of material. An albumen fixative solution was made (Mayer's albumen fixative) (Guyer 1932), using 10 drops of the undiluted Mayer's solution to 30 cc. of water. A few drops of this solution were placed upon a clean slide and to these was added a small amount of the powder to be examined. The mount with the powder well mixed with the albumen solution and smeared over the surface of the slide, was then placed in an oven at 58°C. to 60°C. or allowed to stand in a covered box over night, until dry. The slide could then be run through any of the usual histological routines and gave a permanent preparation.

This mode of mounting, although it destroyed some of the larger particles, was of great value in observing individual cellular staining reactions. It was valuable in staining whole pituitary with Borrel's methylene blue and eosin as the individual chromophobe, acidophilic and basophilic cells could be detected through their characteristic staining reactions. It should be noted here that any solutions which were too strongly acid or alkaline, or too rapid changes from water to high percentages of alcohol (95% and absolute alcohol) caused many of the powder particles to become detached with the consequent destruction of that preparation.

Temporary mounts. Because of the necessity of making numerous mounts of these powders in order to find some of the less frequently occurring elements, temporary mounts were resorted to more often than permanent preparations. Then too, the use of certain reagents for special color reactions or because of their dissolving action on certain tissue elements in the powders, necessitated resorting to temporary mounts.

The author has used a number of solutions or reagents for staining of the powders. A list of those used might be of advantage

to future workers. Any complete text (Lee 1928) on histological technique will furnish the methods for making the stains used.

1. $AgNO_3$—1%.
2. Mallory's phosphotungstic acid-hematoxylin.
3. Aqueous eosin (1%).
4. Saturated alcoholic eosin.
5. Ehrlich-Biondi-Heidenhain Triple stain.
6. Borrel's methylene blue. (Carleton, 1926.)
7. Mann's methylene blue-eosin.
8. Methylene blue (1% aqueous).
9. Acid fuchsin (1% aqueous solution plus 0.1 cc. dilute HCl in 100 cc. water).
10. Picro-carmine (Guyer, 1932).
11. Iodine T. S.
12. Osmic acid—1% aqueous.
13. Gold chloride—1% aqueous.
14. Sudan III—alcoholic.
15. Picric acid (saturated aqueous solution).
16. Picric acid (1% aqueous).
17. Phosphomolybdic acid—1%.
18. Ferric chloride T. S.
19. Weigert's elastic tissue stain.
20. Ponceau S. (Carleton, 1926.)
21. Dahlia or Hoffmann's Violet—(1% aqueous solution).
22. Orcein (Guyer, 1932).
23. Ammonium picrate.
24. Light green in clove oil.
25. Gentian violet in clove oil.
26. Mallory's triple connective tissue stain. (Guyer, 1932.)
27. Delafield's hematoxylin.
28. Heidenhain's hematoxylin.

Certain of these stains were found to have no particular advantage and were discarded in favor of those giving uniformly excellent results. For example, the Ehrlich-Biondi-Heindenhain triple stain was notably capricious; picro-carmine of no great value (except for thyroid) because of its bulk staining characteristics; and Sudan III was discarded in favor of osmic acid.

The author also used a number of other reagents, whose purpose will be mentioned at the proper time. Those most useful were:

1. 10% sulphuric acid.
2. 20% sulphuric acid.
3. Concentrated sulphuric acid.
4. 2% acetic acid.
5. Potassium picrate.

The media used in making temporary mounts of the stained or otherwise treated powders were water, glycerin and Karo for those mounts in which water was present. In certain other mounts, clove oil, a mixture of clove oil and oil of red thyme, cedarwood oil or aniline oil were used.

In staining the powders investigated, the author found that the results secured by using a dilute staining solution for a longer period of time were superior to those obtained using the concentrated stain for a shorter period of time. For example, Borrel's methylene blue if used in the usual method (Guyer, 1932) must be diluted in the proportion of one part of stain to five parts of distilled water. It was found that for staining temporary mounts of the powders, one drop of the stain in twenty drops of distilled water would stain beautifully in one to two minutes. The author wishes to stress the fact that any time intervals which are stated as having been used, are those which were found by many trials to give the most success. The variation in the size of the individual particles, as well as the differences in staining reactions of the various elements of a single powder made it difficult to use a uniform method for each powder. Likewise if the stain was to be followed by dehydration in alcohol, clearing and mounting in balsam, it was found necessary to overstain the powder if the stain was alcohol soluble. In smear preparations which were to be stained with Borrel's methylene blue and eosin the author always overstained with eosin and Borrel's because of the rapid destaining in alcohol during the dehydration process. Even with the greatest care in modifying the staining times, not all elements of the powders were stained with equal intensity. As mentioned before, there was a great variation in the size of the individual particles of the powder, so that while the smaller particles were stained properly, the larger particles were only stained at the periphery. If these larger par-

ticles were successfully stained, the smaller bits of tissue were too deeply colored as a general rule.

Certain of the stains used in this study should be mentioned for their value in routine work or because of some special staining properties which they have demonstrated.

Borrel's methylene blue. This stain was used more extensively in the study than the other stains. In combination with aqueous eosin (1%), it gave excellent results. In the proportion of one part of stain to twenty parts of water, the stain was used on all of the powders in routine examinations by means of temporary mounts. The powders were stained for two minutes in this dilution followed by a washing with water and rapid staining in very dilute eosin made from the 1 per cent. aqueous solution. After a second washing in water, the powders were mounted in glycerin or Karo and could be set aside until ready for study.

Delafield's hematoxylin and eosin. Delafield's hematoxylin was used both undiluted and diluted. The diluted stain was more effective in staining the powders in bulk than the more concentrated stain. The concentrated stain followed by alcoholic eosin was used for smear preparations and for staining those sections which had been successfully mounted in paraffin. Nuclear differentiation was secured, but there was a tendency for the entire tissue to take up the hematoxylin stain so that the sections thus stained showed dark purple nuclei in a reddish purple background.

Mallory's phosphotungstic acid hematoxylin. This stain was diluted with one part of water when staining the powders in bulk. In the smear method as with the other stains, the stain was used undiluted. It was useful in revealing fibrous tissue, nerve fibers and neuroglia.

Methylene blue. This stain was used as a 1 per cent. aqueous solution. Because of the rapidity and depth of the staining it was usually diluted with three to four times its volume of water. Because of the success secured with Borrel's methylene blue, the 1 per cent. solution of methylene blue was not used frequently.

Silver nitrate—1%. The powder to be examined was allowed to remain in silver nitrate for five minutes, washed with distilled water and mounted on a slide in water. The slide was then exposed to bright sunlight for one to two minutes. After washing the powder again, it was mounted in glycerin.

Iodine solution. A very weak aqueous iodine solution was used in testing for certain adulterations.

Osmic acid. A 1 per cent. solution was made as a stock solution. This was usually diluted with an equal volume of water before using. Osmic acid was found useful in detecting fat, nerve fibers, and the colloid material in whole pituitary.

Gold chloride. A 1 per cent. solution was used in all cases, reduction being secured by means of a 2 per cent. formic acid solution or sunlight until the tissues were purplish in color. After carefully washing with water, the powder was mounted in glycerin. Several mounts of whole pituitary after staining in gold chloride were mounted in equal parts of 20 per cent. sulphuric acid (by volume) and glycerin. A few of these mounts were very successful, showing cellular outlines far better than any other methods used. It must be stated, however, that the author has been unable to secure consistent results with this method, some mounts being successful and others of no relevant value.

Ammonium picrate. This solution was made by adding concentrated ammonium hydroxide to picric acid until neutral. Powders mounted in ammonium picrate were stained uniformly yellow, but any fragments of striated muscle which were present were at once apparent. The cross striations were particularly prominent. Likewise, elastic fibers stood out as wavy bands when mounted in ammonium picrate.

Picric acid—1%. This was found useful following staining by means of acid fuchsin as collagen was stained red while muscle tissue, epithelial tissue and other tissues are yellow.

Ponceau S (Curtis's substitute for Van Gieson). Ponceau S was made according to the method in Carleton (1926) and was found to be of some value in demonstrating collagenous tissue. It was not uniformly successful, however, as Mallory's triple connective tissue stain gave somewhat better results.

Mallory's triple connective tissue stain. In the use of this stain the usual histological procedure was modified. To the usual stain an equal volume of distilled water was added and the resulting dilution used with the dry powders. In staining smear preparations, the usual method using acid fuchsin, phosphomolybdic acid and Mallory's stain was used (Carleton, 1926).

The mounting medium most frequently used was glycerin. Glycerin mounted slides may be kept for some time without the necessity of sealing the mounts. The slight clearing action of the glycerin was also found helpful in making examinations. Karo was used in a few instances as a mounting medium, but was found unsatisfactory because of the instability of stain in such a mount. In routine examinations where the mounts were not to be kept for any length of time, the powders were sometimes mounted in water, in a very dilute solution of the stain used or in the reagent being used at that time. Clove oil and cedarwood oil were also used for temporary mounting in a few instances. The clearing properties of these oils were of value in examining bits of the tissue which were quite thick. Powders mounted in cedarwood oil without a coverslip could be examined with the oil immersion lens with little difficulty.

In the paper published in 1935 by Professor Youngken (1935) he points out that powdered, desiccated whole ovary may be distinguished from ovarian residue by the use of sulphuric acid and old ammonium polysulphide. He found that when one gram of ovarian residue was mixed with 1 cc. of sulphuric acid a yellowish green or fig color was produced, which upon the addition of about 0.5 cc. of old ammonium polysulphide was immediately changed to a white color. He (1933) also states that a paste made by mixing 1 gram of whole ovary with water was turned deep red to reddish brown by the addition of 0.5 cc. of sulphuric acid. The author has made many attempts to verify these results but has met with no success. In a communication sent to the National Formulary Committee on Revision in 1935, the author pointed out that sulphuric acid gave a marked reddish color with powdered ovary in one test of a series of more than twenty tests. However, the reddish color was produced with powdered desiccated thyroid under the same conditions in several tests. Whole pituitary gave a reddish purple color, but this color was not intense nor characteristic. In the case of corpus luteum (using new samples), a slight greenish tinge was noticed, but this color was masked by the brown from the charring due to the sulphuric acid. In more recent attempts to secure these color reactions of Youngken the author utilized concentrated sulphuric acid, 20 per cent. sulphuric acid (by volume) and 10 per cent. sulphuric acid (by weight) with all the powders. In all cases the reactions with ovarian substance and ovarian residue were negative.

Though the yellowish green color or fig color produced by the addition of sulphuric acid to powdered ovarian residue could not be secured, the author tried the addition of old ammonium polysulphide in an attempt to secure the white color. The white color reported by Youngken was produced, but when the same test was also tried with whole ovary, corpus luteum and whole pituitary, as well as on a control of plain sulphuric acid, a white color was produced in all cases. This white color is not due to any particular chemical combination formed by the action of sulphuric acid on the powders or their active principles, but is due rather to the reaction of ammonium polysulphide with sulphuric acid with the production of a white precipitate of finely divided sulphur.

David (1934) reported a color reaction for trihydroxyestrin in which 30 y trihydroxyestrin are heated with concentrated sulphuric acid in a water bath, cooled and diluted with water. To this solution is added arsenic acid with the production of a clear blue solution. It was thought that by using the powdered ovarian substances in gram quantities, sufficient active principle might be present to give the reaction. To the powder was added 2 cc. of concentrated sulphuric acid. The tubes were placed in a boiling water bath for two minutes and then, while cooling, diluted with 1.6 cc. of distilled water. To this solution were added sixteen drops of arsenic acid. This reaction was negative for all powders and controls and all modifications of technique were likewise negative.

Tri-hydroxy-oestrin (oestriol or theelol) when heated with concentrated sulphuric acid gives an orange color with a greenish fluorescence. With this fact in mind the following experiment was conducted. Five test tubes were used. Into the first tube a 500 milligram sample of desiccated whole ovary was placed; into the second tube, an equal amount of desiccated corpus luteum; and equal amounts of desiccated thyroid, ovarian residue and whole pituitary in the remaining three tubes. To each tube were added 5 cc. of distilled water. After standing for ten minutes, 2 cc. of concentrated sulphuric acid were added to the solution and the tubes placed in a water bath at 95°C. to 100°C. The tubes were allowed to remain in the water bath for a few minutes and then removed to a test tube rack. Upon settling the supernatant liquid had assumed a color. In the case of whole ovarian substance, the supernatant fluid was orange brown in color. Ovarian residue possessed a similar color, perhaps of

a trifle lighter shade than that from whole ovary. The supernatant fluids from corpus luteum, whole pituitary and thyroid were all brownish red, that from thyroid being redder than the preceding pair. No fluorescence was noted in any of these solutions.

Another series of experiments were run using absolute alcohol instead of distilled water. After allowing the powders to stand for ten minutes in the absolute alcohol, the powder residue was filtered off. To the alcoholic filtrate were added 2 cc. of concentrated sulphuric acid. In the case of whole ovary, the addition of sulphuric acid gave an orange color slightly more pronounced on a gentle heating, which upon standing developed a greenish fluorescence. Ovarian residue gave a light orange color, but did not develop the fluorescence. None of the other powder extracts from thyroid, whole pituitary or corpus luteum developed this orange color. The colors in the cases of the last-mentioned glands were too nearly alike to be stated as characteristic.

Before discussing the histological constituents found in the powders examined, something should be said concerning the color and odor of these products. It is the writer's opinion that little emphasis should be placed on either color or odor, because of the variations among various samples of powders and particularly because of the human element, that is, the discordance of opinion in judging the color or odor of the same sample. No two sets of glandular products will possess exactly the same color. For example, the color of ovarian substance will be dependent upon feeding, the season of the year at which the gland is removed and the species of animal used for the product.

Histological Constituents of the Powders

Powdered Desiccated Whole Ovary

Occasionally one found bits of the mature Graafian follicles. Rarely there was seen a portion of the follicle showing a part of the stratum granulosum with the theca externa and interna still intact (Figure 2, Plate A). Still more rarely (the author found only one example in over fifty mounts) one may find an entire ovum still attached to the theca folliculi, with the radially arranged cells surrounding the ovum (the corona radiata) well marked. The mount in which the writer found this example had been stained in bulk; in an embryological dish with Delafield's hematoxylin (diluted with an equal volume of distilled water) followed by aqueous eosin. The

nucleus of the ovum was clearly seen as a rounded dark purple body. The outlines of the cells of the corona radiata were evident, but the nuclei were only partially stained. The rounded nuclei of the cells of the stratum granulosum were quite clear.

In all mounts were found portions of the theca folliculi varying in size. These in most instances had a curved outline with fine wavy fibers running parallel with the curvature. These fibrils were stained pink with eosin, red with acid fuchsin and blue with Mallory's stain. Stained with Delafield's hematoxylin and eosin, these fragments showed elongated, ovoid nuclei staining deep purple. With Borrel's methylene blue and eosin, they stained deep blue against a bright pink background.

In all preparations a large amount of connective tissue was found, not only that surrounding the growing and mature follicles and the lutein cells, but also that forming the groundwork of the ovarian stroma. There were found bits of the stroma in which were imbedded many cells having a fusiform shape, with elongated, oval to rod-shaped nuclei. These cells had a marked resemblance to smooth muscle cells except that they had no fibrils in the cytoplasm.

Rarely, there were seen a few tall columnar cells, usually with one or both ends curved and pointed, lying parallel one to the other. The nuclei of these cells were small and stained uniformly purple with Delafield's hematoxylin or blue with Borrel's methylene blue.

Occasional masses of lutein cells were seen. These cells were rounded or polygonal in shape with a small rounded nucleus staining light purple and the cytoplasm a faint purple to pink with Delafield's hematoxylin and eosin (Figure 1, Plate A). With Borrel's methylene blue and eosin, the nucleus stained a bright blue and the cytoplasm a deep pink, with occasional cells showing a purplish cast to the cytoplasm. In effective mounts some of the lutein cells showed distinct fat droplets, stained black with osmic acid. The masses of lutein cells were yellowish in color in water mounts or in clove oil mounts, and frequently the cells would stand out as yellowish masses in mounts where the stain was not so heavy. In one smear preparation stained with Delafield's hematoxylin and eosin, lutein cells were found in which large irregular spaces were present, surrounded by a faint purple cytoplasm in which a few very small, dark purple granules were seen.

In the majority of mounts, there were small fragments of vessels. These were either pieces of larger vessels from the medulla of the ovary or bits of smaller vessels and capillaries. The capillaries often showed walls stained very light blue with Borell's methylene blue. In some preparations, the capillaries were unstained, merely appearing as short hyaline-like segments with sharply broken ends. The larger vessels were rarely seen in their entirety. Infrequently one could find a cross-sectional view with a portion of the endothelium remaining, the internal elastic lamina appearing bright and highly refractive and a segment of the tunica media still intact. The nuclei of the smooth muscle cells of the tunica media were clearly defined when stained with either Borell's methylene blue and eosin or Delafield's hematoxylin and eosin. More frequent than the cross-sectional views of these vessels were the fragments of the muscular tunic of the larger vessels of the medulla which one finds in smear preparations. These were often crushed and broken, but when stained the cylindrical or rod-shaped nuclei with rounded ends and the parallel position of the cells made them unmistakable.

In smear preparations stained with Borell's methylene blue and eosin, one occasionally found small rounded cells, smaller than lutein cells, which possessed bright blue nuclei and small granules stained bright red, scattered throughout the cytoplasm (Figure 4, Plate A). A few of these cells had one end drawn into a point or beak. These were cells from the ovarian stroma. These cells also were found to show the same kind of granules stained red with Bordeaux B.

In the majority of mounts there were found a few small sections of unmyelinated nerve fibers staining light blue with Borell's methylene blue. In 1 per cent. methylene blue, these fibers stained deep blue. Rarely, with Borell's methylene blue one could find short segments of myelinated fibers in which the axis cylinder and neurilemma had been stained blue, but the myelin sheath was unstained (Figure 5, Plate A). At times there were found small bits of the tissue with small unmyelinated fibers imbedded therein (Figure 3, Plate A).

The writer found numerous fragments of connective tissue from the cortical and medullary areas (Figure 6, Plate A). When the powder was mounted in potassium picrate, the elastic fibers stood out as wavy, yellowish strands. In Mallory's connective tissue stain, these masses stained blue. In 2 per cent. acetic acid, the collagenous fibers were swollen and easily distinguishable while the elastic fibers were untouched.

Hyaline masses varying in size and shape were noted in approximately every second mount. In the unstained mounts, these usually were seen as irregularly rounded masses of a light yellow color. They were stained pink with eosin and bright red with acid fuchsin. Rarely they would stain black with silver nitrate (1%).

Rarely there were observed small bits of striated muscle, plainly seen in water mounts and more sharply defined by mounting in ammonium picrate.

Powdered Desiccated Corpus Luteum

Mounts of this powder contained numerous isolated lutein cells usually ovoid to polyhedral in shape (Figure 1, Plate A). They possessed in many instances a prominent centrally placed nucleus. In Borrel's methylene blue and glycerin mounts, these cells were stained from light blue to greenish blue. In unstained preparations, they often showed cytoplasmic granules. With smear preparations using Borrel's methylene blue followed by eosin, the staining was somewhat varied; the nucleus stained bright blue and the cytoplasm varied from a bright pink and slightly granular to a light purple, clear and homogeneous.

Masses of lutein cells occurred in all mounts, usually with more or less connective tissue attached to the mass. In some instances, cells found in the theca, which was still attached to the luteal mass, were filled with a yellowish pigment, so that the entire mass of connective tissue had a yellowish, granular appearance. The connective tissue had the same staining reactions as those mentioned under whole ovary.

Occasionally one would find clear, hyaline fragments in unstained mounts. These were stained light blue with Borell's methylene blue and dark brown with silver nitrate (1%).

In several glycerin-mounted specimens of this powder treated with Borell's methylene blue, there were noted small angular fragments of a hyaline nature which appeared almost crystalline. They were stained a bright blue with Borrel's whereas the remainder of the tissue elements had for the most part a greenish blue cast.

As in whole ovary, one found fragments of capillaries, occasional bits of larger vessels and rarely an unmyelinated nerve fiber.

Powdered Desiccated Ovarian Residue

As Youngken (1933, 1935) pointed out, the same elements exist in powdered ovarian residue as in powdered whole ovary with a relatively smaller amount of lutein material, that is, lutein cells. To the writer, the distinguishing feature of ovarian residue was the relatively greater amount of connective tissue, present in every mount, compared to similar mounts of ovarian substance.

Rarely one would find lutein cells and small masses of lutein material, but these were found only after careful searching, whereas the other elements were immediately apparent. Masses of connective tissue from the medulla (Figure 6, Plate A), bits of the theca folliculi (Figure 2, Plate A), fragments of capillaries and larger vessels and less frequently bits showing immature ova with the surrounding stroma were encountered. Curtis's substitute for Van Gieson or Mallory's triple connective tissue stain were found of value in bringing out the large percentage of connective tissue in this powder. Rarely one could find small bits of striated muscle. These were seen in about one mount in ten.

As the author has pointed out earlier in this paper, the use of concentrated sulphuric acid and ammonium polysulphide on this powder could not be employed as a means of distinguishing desiccated ovarian residue from whole ovary. It was found difficult to make a paste by adding 1 cc. of concentrated sulphuric acid to one gram of ovarian residue. It required at least 2 cc. in order to secure a pasty mass.

Powdered Desiccated Thyroid

The feature which proved most striking in mounts of thyroid were the many fragments of colloid varying in shape from those having rounded margins to others of a quite angular nature. These fragments varied from colorless to pale yellow. The particles likewise were inconstant in their structure. Many pieces were clear and homogeneous, others heavily granular to lightly granular and still others contained many vacuoles of varying sizes (Figure 1, Plate B). Some few bits of colloid were found containing scattered cells. The staining reactions of this colloid material also showed wide differences. In smear preparations stained with Borrel's methylene blue and eosin, the colloid was stained as a rule a dark purple to dark blue, although in certain cases the stain was quite mottled showing vary-

ing shades from light blue through to purple. In temporary mounts with Borrel's methylene blue, the colloid assumed a very light blue color. In the 1 per cent. silver nitrate method, the colloid stained in varying shades of brown from a light yellow brown to occasional bits showing a deep reddish brown. With picrocarmine, the colloid stained bright yellow, whereas the majority of the other fragments (epithelial nature) stain red. One per cent. eosin was also found to stain the colloid powerfully.

Occasionally there were noted small fragments of colloid nature, much smaller than the usual pieces of colloid seen in these mounts. These were deep yellow to yellow-brown in color in unstained preparations and apparently did not take the stain when stained in Borrel's methylene blue. When acid fuchsin was used following Borrel's methylene blue, these fragments could still be found showing their characteristic yellow-brown to deep brown color.

In all mounts were found many bits of follicular tissue varying in size and shape. Occasional fragments were seen with the epithelium lining the follicular lumen and the delicate connective tissue stroma still intact outside of the follicle wall. The nuclei of these follicle cells were small and rounded. With Borrel's methylene blue, they stained deep blue, the cytoplasm a lighter blue. Smear preparations stained by means of Borrel's methylene blue and eosin had the follicular nuclei stained deep purple to blue and the cyptoplasm pink (Figure 3, Plate B).

At times there were found bits of follicular tissue seen in surface view (Figure 2, Plate B). Here with cell outlines visible the cells were seen to be fairly large, irregularly polygonal to rounded with a small rounded nucleus. Along one edge or running between these cells, could be defined small connective tissue fibers and rarely a capillary was seen with a few of the follicular cells still adherent to the sides. In mounts of Borrel's methylene blue followed by acid fuchsin, the connective tissue stood out as bright red fibrils, while the nuclei of the follicular tissue were deep blue (Figure 4, Plate B).

In suitable mounts, there were observed a few isolated rounded cells with a small rounded nucleus, usually eccentrically placed, staining deep blue with Borrel's methylene blue; the cytoplasm, light blue with the same stain. It has been assumed that these cells were probably a type of follicular cell.

In most preparations, there were noted small segments of unmyelinated nerve fibers stained light blue with Borrel's methylene blue and appearing as highly refractile threads in unstained mounts.

The fragments of connective tissue fibers usually show a rather straight or parallel course in distinction to bits found in whole ovary and ovarian residue, where they are frequently quite large and occur in curved segments. The portions of connective tissue found in powdered thyroid occasionally have parts of the follicular epithelium still attached. When carefully stained with Borrel's methylene blue, the small elongated nuclei of the connective tissue were well defined.

Rarely one found fractions of striated muscle perhaps slightly more frequent in occurrence than in ovarian substance.

Small portions of capillaries having a clear hyaline appearance with serrated ends and frequently unstained in stained mounts were noted. Fragments of larger vessels were rarely seen.

It might be noted that potassium picrate was found of advantage in preparing mounts to show the follicular structure. Figure 5. Plate A was prepared by this method. The inter-follicular connective tissue remains untouched while the epithelial tissue is disintegrated.

Powdered Desiccated Whole Pituitary

In temporary mounts of whole pituitary the author found many clumps of cells with adherent connective tissue. These cells were rounded to polyhedral in shape and show small rounded nuclei staining deep blue with Borrel's methylene blue. Occasionally one might see small capillaries or arterioles running through a mass of cellular material (Figure 2, Plate C). These cells were frequently arranged in cords of varying size and shape. It was often difficult to discern cellular structure in these masses, but when stained with Borrel's methylene blue or Delafield's hematoxylin, nuclei were observed arranged in a more or less linear fashion in the cord-like structure.

Numerous smaller clumps of cells (Figure 8, Plate C) and individual cells were noted. These cells were of the same types as those in the larger masses spoken of above. It was noted that at least two types of cells could be distinguished from their staining characteristics. In one type, the nucleus was stained deep blue and the cytoplasm pink with Borrel's methylene blue and eosin. In the second type, the nucleus also stained deep blue, but the cytoplasm stained a pale blue, with the above mentioned stain.

In smear preparations of whole pituitary, the same types of cells were noted and in addition at least one other type was seen. This third type of cell usually showed a deep blue nucleus and a small ring of faint pinkish to faint purple cytoplasm when stained with Borrel's methylene blue and eosin.

The above indicated cells are the chromophile and chromophobe cells of the anterior lobe (pars distalis) of the pituitary. Those cells showing a bright red cytoplasm were the so-called alpha-cells or acidophilic cells of the pars distalis. When these cells were observed under oil immersion, they were found to have large red granules scattered through the cytoplasm. Examination of cells showing a faint blue cytoplasm disclosed the presence of small blue granules in the cytoplasm, establishing these cells as the beta-cells or basophilic cells of the pars distalis. The third type, spoken of in the preceding paragraph, which had only a small amount of cytoplasm in comparison to the size of the nucleus, has been considered to be the chromophobe cells of the pars distalis.

The author wishes to point out that with smear preparations other types of cells were found which varied in some degree from the cell types listed here, but since their differences were due rather to position at the time of observation and mechanical interference (such as, crushing, etc.), they have not been listed as definite cell types. A type of cell was sometimes noted in a smear preparation which contained an elongated to rounded vesicular nucleus surrounded by a fairly large amount of cytoplasm. These cells were more rarely seen than the preceding types and were taken as coming from the pars intermedia of the pituitary.

Now and then preparations showed colloid fragments of varied sizes and shapes. All stained pink with eosin and the majority of these bits stained black to dark brown with silver nitrate 1 per cent. Infrequently one observed colloid inclusions (Figure 5, Plate C) or colloid masses surrounded by cells (Figure 6, Plate C).

In the majority of mounts, one sees fragments of the pars nervosa (Figure 4, Figure 10, Plate C), in which, when stained with Borrel's methylene blue, were observed small nerve fibers with bulbous ends and small fusiform or elongated cells with one or more delicate processes extending from one or both ends. These latter cells, sometimes stained very faintly purple with Borrel's methylene blue. In one mount using gold chloride (1%) and mounting in 20 per cent.

sulphuric acid, these cells were clearly defined against a brownish, granular background, as faint blue neuroglia cells. Small rounded cells without processes and containing a large elongated deeply staining nucleus were sometimes noted in the portions from the pars nervosa. The groundwork or matrix of these bits from the pars nervosa, mounted in Borrel's methylene blue, were either unstained or showed a faint bluish granulation with here and there very tiny fibrils staining deep blue.

With silver nitrate (1%) staining, it was possible to demonstrate here and there neuroglia cells staining black and showing many fine irregular branching processes. These could also be detected when stained with methylene blue, taking then a deep blue color (Figure 9, Plate C).

Here and there one could find bits of unmyelinated nerve fibers staining light blue with Borrel's methylene blue and grey with osmic acid (1%). In smear preparations, stained with Borrel's methylene blue and eosin, it was at times possible to find unmyelinated fibers running through bits of the tissue. They were stained deep blue, whereas the surrounding bit of tissue was stained pink.

Rarely a fragment of myelinated nerve fiber could be picked out in these mounts. On one slide a myelinated fiber was observed, in which the neurilemma and axis cylinder had stained blue with Borrel's methylene blue and the myelin sheath had remained unstained (Figure 3, Plate C).

Very infrequently, the writer found small rounded cells stained deep blue to purple with iodine.

In all preparations there were seen capillaries, but the fragments of larger vessels were not as frequent as in whole ovary. In one mount the author found a small arteriole with the muscle cells of the media markedly prominent (Figure 1, Plate C).

Rarely one found fragments of the capsule of the gland. In one mount, a portion of the pia mater still remained adherent to a portion of the glandular capsule (Figure 7, Plate C).

Summary and Conclusions

The author has presented those more important histological elements he found in certain powdered endocrine glands, viz: ovarian substance, corpus luteum, ovarian residue, thyroid and whole pituitary.

In carrying out this study, certain procedures were found helpful

in examining these powders. The methods the writer found most helpful were the watch glass method, making use of embryological staining dishes and the smear method. These methods are both useful in making permanent preparations. The watch glass method may also be used in staining when making temporary mounts, instead of staining directly on the slide.

Those stains and reagents which the author has found through frequent use to give the best results are Borrel's methylene blue in combination with eosin, methylene blue and eosin, silver nitrate (1%), acid fuchsin and picro-carmine, gold chloride 1 per cent., osmic acid 1 per cent., ammonium picrate, Mallory's triple connective tissue stain, Delafield's hematoxylin and Ponceau S (Curtis's substitute for Van Gieson). The author found 20 per cent. sulphuric acid, 2 per cent. acetic acid, and potassium picrate of much assistance in bringing out certain features of the powders. (See text.)

As pointed out in the text, better results were secured in bulk staining by using a dilute solution of a stain. Because of variation in the size of powders and the difference in their reactions to the same stains it was not possible to state definite staining times. In bulk staining, one to two minutes in the dilute solution was generally sufficient with the following stains: Borell's methylene blue, Delafield's hematoxylin, methylene blue, acid fuchsin, Mallory's triple connective tissue stain, and Ponceau S. Three to five minutes were adequate for osmic acid (0.5%), silver nitrate (1%) and gold chloride (1%). The powders were generally mounted in weak picro-carmine and ammonium picrate when these reagents were used.

In staining smear preparations it was usually found possible to use standard methods. The writer found that the staining times varied with the powder to be stained and the condition of the smear.

The author has pointed out the histological features of those powders studied, as well as the more striking staining reactions of certain portions. In order to add to the value of the study, the author wishes to point out the salient features of each powder for diagnostic purposes.

Powdered Whole Ovary

There were found to be four things of diagnostic value in this powder, (1) the presence of ova, either immature or developing; (2) the presence of bits of the theca folliculi, with or without

granulosa cells still adherent; (3) lutein cells, in masses or solitary; (4) ovarian stroma, with its stellate connective tissue cells and rounded or beaked cells, the latter sometimes acidophilic.

Powdered Corpus Luteum

Corpus luteum was characterized by (1) the great number of lutein cells in masses or isolated; (2) the lack of ova; and (3) the presence of the thecal walls with lutein cells sometimes adhering thereto.

Powdered Ovarian Residue

This powder, the writer found, was readily distinguishable because of (1) the relatively large amount of connective tissue; (2) the absence of or sporadic occurrence of lutein cells; and (3) the presence of ova and stromal cells.

Powdered Thyroid

Powdered thyroid was recognized through (1) the presence of the follicular tissue and (2) the large amount of colloid.

Powdered Whole Pituitary

This powder presented certain difficulties, but by the use of the smear method and careful observation one could detect (1) the three types of cells characteristic of the pars distalis, the chromophile cells (alpha and the beta types) and chromophobe cells; (2) the presence of bits of tissue showing a cordlike structure; (3) small pieces of colloid and (4) neuroglia cells in combination with neuraxons.

It will be noted that colloid was found in all of the powders, but the amount found in powdered thyroid so far exceeds that found in the other powdered products that it could be used as a diagnostic feature. In addition, the structure of the colloid from the thyroid with the vacuoles and occasional cells found in its substance, lessened the possibility of misinterpretation.

The histological features of five of the powdered desiccated glandular products have been studied and the descriptions given. Those features of diagnostic value have been listed in the hope that they may be of some value to future workers in this field.

For the samples with which the author worked, the test for theelol (see text) was characteristic of whole ovary only and suggestive of the possibility of its use as a test for identity.

The author wishes to express his gratitude to the many friends who have been of material assistance in preparing this study, particularly to:

Dr. Marin S. Dunn, without whose assistance and interest this study could not have been made.

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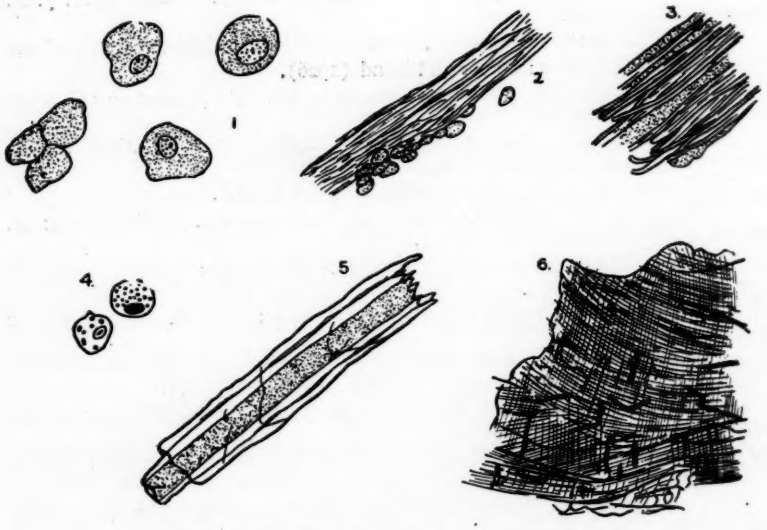

PLATE A. POWDERED DESICCATED WHOLE OVARY.

Fig. 1. Lutein cells.

Fig. 2. Portion of theca folliculi showing cells of the stratum granulosum still adhering.

Fig. 3. Unmyelinated nerves in ovarian tissue.

Fig. 4. Ovarian, stroma cells. (Notice beaked cell.)

Fig. 5. Portion of myelinated nerve.

Fig. 6. Connective tissue from the medulla.

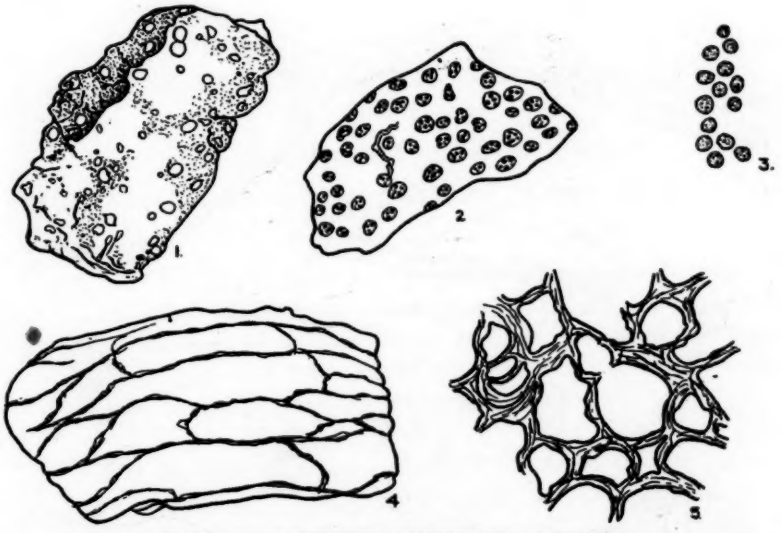

PLATE B. POWDERED DESICCATED THYROID.

Fig. 1. Colloid fragment showing granules, a few cells and vacuoles.

Fig. 2. Follicle wall, showing nuclei of follicular cells.

Fig. 3. Isolated follicular nuclei showing nucleoli.

Fig. 4. Follicles and connective tissue (lateral view).

Fig. 5. Follicular tissue (cross-sectional view).

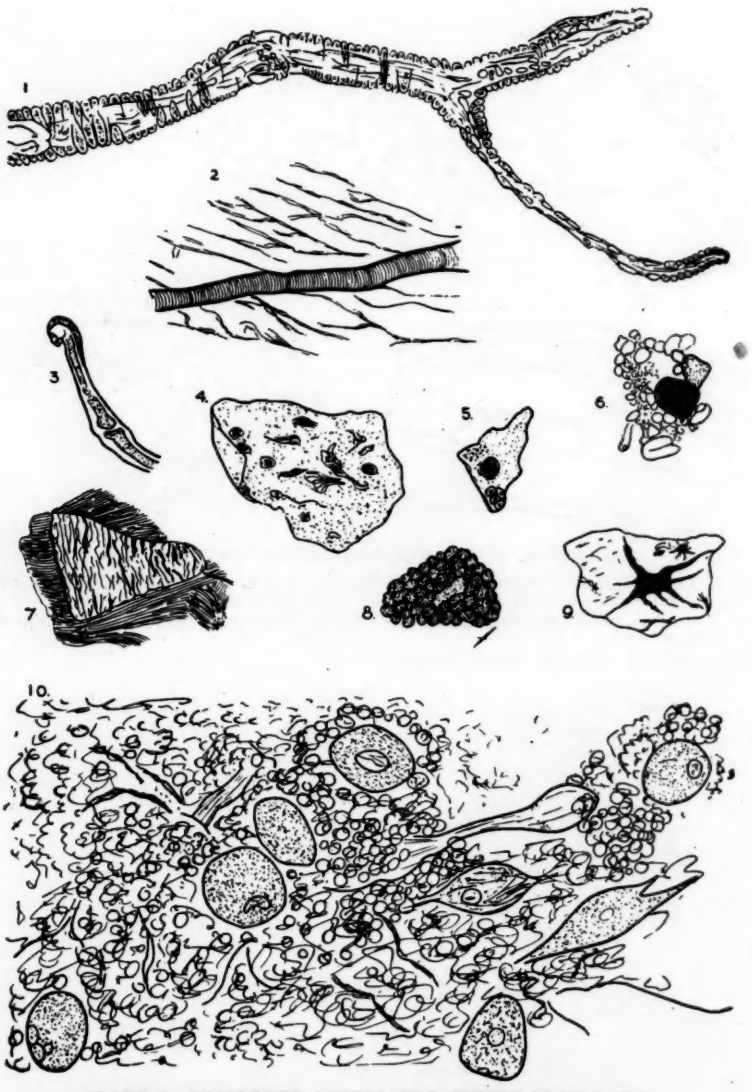

PLATE C. POWDERED DESICCATED WHOLE PITUITARY.

Fig. 1. Small arteriole.
Fig. 2. Fragment from anterior lobe (pars distalis) showing capillary in tissue.
Fig. 3. Myelinated nerve.
Fig. 4. Fragment of pars nervosa.
Fig. 5. Colloid inclusion in fragment of pars nervosa.
Fig. 6. Small follicle, showing colloid.
Fig. 7. Portion of fibrous capsule.
Fig. 8. Group of cells from pars distalis.
Fig. 9. Isolated glial cell.
Fig. 10. Portion of pars nervosa showing cell types.

THE OFFICIAL MEDICINE DROPPER OF THE U. S. P. XI

By S. E. Harris and W. G. Christiansen*

THE official medicine dropper is defined on page 455 of the U. S. P. XI as follows:

> "The official medicine dropper shall have its delivery end 3 millimeters in external diameter, and shall be adjusted to deliver 20 drops of distilled water, weighing 1 Gm. at a temperature of 15°C. A tolerance of 10 percent above or 10 percent below the delivery specifications is permitted."

From the theoretical considerations discussed in this paper it appeared that the diameter specified would not deliver drops of the required weight. Accordingly experiments were undertaken to test this point and to find what diameter would be necessary. A few droppers having delivery ends 3 mm. in outside diameter were constructed and found to deliver drops weighing close to the lower limit of 0.045 gm. Tests on other droppers showed clearly that considerable variations occurred in the weights delivered by a single dropper in a series of experiments (Table III below). Consequently, if the average weight delivered by a dropper is close to either limit, the drops delivered under ordinary conditions will fall outside the official tolerance about 50 per cent. of the times used.

The weight of drops delivered from small orifices was first determined by Tate (1). He stated that the weight of the drop falling from a tube was proportional to 1), the diameter of the tube 2), the surface tension of the liquid, and was diminished by rise of temperature. The first two statements combined gave rise to the familiar expression known as Tate's law:

$$Mg = 2 \pi rT \quad \ldots\ldots\ldots\ldots\ldots \quad I$$

where M is the mass of the drop, g is the acceleration of gravity, r is

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the radius of the orifice and T is the surface tension of the liquid, all expressed in suitable units.

Numerous investigators were unable to confirm the law of Tate and the truth of equation I. Tate used orifices ground to a sharp edge, while later workers used flat ended tubes. Both devices were intended to prevent the drops from extending up the vertical sides of the tube from which they were formed. The laws of Tate as stated in his original paper were shown by Livingstone, Morgan and Stevenson (2), to be true under the conditions used by Tate, these authors maintaining that the expression of the first two laws by equation I gave to them a meaning never intended by Tate.

Rayleigh (3), in a series of careful experiments, showed that a close approximation to the drop weight was given by the empirical relation—

$$Mg = 3.8\ rT \quad \ldots\ldots\ldots\ldots\ldots \quad II$$

When r is 0.15 cm. as required by the U. S. P. definition, and taking T as 73.2 dynes/cm. at 15°C., equation II yields a value of 0.0425 gm. for M. This is below the lowest permitted weight for the official drop, and is similar in magnitude to our value found by experiment. By making M equal to 0.05 gm. and calculating r, we find that it needs to be 0.176 cm. and the outside diameter required is therefore 3.5 mm. With the tolerances permitted in the U. S. P. XI definition the upper and lower limiting weights are 0.055 and 0.045 gm. From equation II at 15°C. these require tip diameters of 3.86 and 3.18 mm. respectively.

Rayleigh stated that the rate of formation of the drop affected the weight, and this was later studied by Guye and Perrot (4), who found that rapidly formed drops were heavier than slowly formed drops. Rayleigh, in discussing Tate's law stated that dynamical considerations must enter into the formation of the drop at the instant of separation from the orifice and thus invalidate the relations deduced by Tate from purely statical considerations. Guye and Perrot (5), photographed the falling drops and proved that they were not

detached from the rim of the orifice but from a neck which formed in the liquid, Fig. 1, a and b.

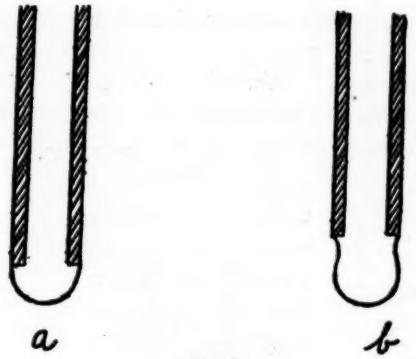

Fig. 1

This also suggests that the analytical expression of Tate's law, equation I, cannot give an accurate measure of the drop weight.

Our experiments showed that with a delivery tip diameter 3.5 mm., as suggested by the calculation from Rayleigh's formula, equation II, the drops are heavier than 0.05 gm., and that when the outside diameter is 3.3-3.4 mm., the drops are consistently within the prescribed limits.

TABLE I.

Drops delivered at 15°C. from several droppers all having external diameter of 3.5 mm.

Dropper No.	Wt. of 20 drops	Wt. of 1 drop
4	1.269 gm.	0.063 gm.
6	1.024	0.051
5	0.997	0.045

TABLE II.

Successive deliveries from a single dropper, No. 5 above, external diameter 3.5 mm.

Weighing No.	Wt. of 20 drops	Wt. of 1 drop
1	1.025 gm.	0.051 gm.
2	1.033	0.052
3	1.014	0.051
4	1.095	0.055
5	1.097	0.055
6	1.129	0.056

TABLE III.

Drops delivered from several droppers with varying diameters.

Dropper No.	Diameter	Wt. of 20 drops	Wt. of 1 drop	Wt. of 1 drop calculated by Equation II
7	3.44 mm.	1.024 gm.	0.051 gm.	0.049 gm.
8	3.47	1.035	0.052	0.049
9	3.35	0.983	0.049	0.047
10	3.39	0.933	0.047	0.048
11	3.33	1.001	0.050	0.047
12	3.40	0.961	0.048	0.048
13	3.55	1.038	0.052	0.050
14	3.40	1.026	0.051	0.048
15	3.51	1.033	0.052	0.049
16	3.60	1.111	0.055	0.051

TABLE IV.

Successive deliveries from a single dropper, No. 10 of Table III—external diameter 3.39 mm.

Weighing No.	Wt. of 20 drops	Wt. of 1 drop
1	1.061 gm.	0.053 gm.
2	0.971	0.049
3	1.044	0.052
4	1.021	0.051
5	0.999	0.050
6	1.031	0.052
7	1.063	0.053
8	1.016	0.051
9	0.999	0.050
10	1.031	0.051

In all of the above experiments the droppers were made by drawing out glass tubing to form tips of the required diameter and having walls about 0.7 mm. thick. The end of the tip was fire polished and the whole dropper cleaned with hot bichromate-sulfuric acid. The droppers were held in a vertical position and the drops slowly expelled by squeezing a rubber bulb attached to the tube. This

procedure simulates the conditions under which they would be used in actual practice, and the results are therefore of a degree of accuracy which reasonably could be expected in ordinary use.

In the preliminary experiments the greatest variations were introduced by the state of cleanliness of the tip. The presence of minute traces of grease always led to the formation of smaller drops. This is an obvious result when it is considered that the drop is supported by the surface tension of the water.

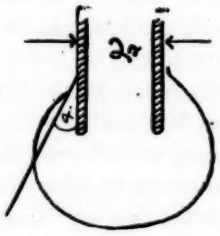

Fig. 2

Figure 2 shows the drop just about to fall from a greasy tube. The drop is supported by the vertical component of the surface tension at the circle of contact. This vertical component is $2 \pi r T \cos \alpha$ and is a maximum when $\cos \alpha$ is 1; that is, when α is zero. α is only zero when the liquid wets the tube. For all other values of α, $\cos \alpha$ is less than 1, and so the weight of the drop which is supported is smaller.

Summary

The official dropper of the U. S. P. XI has been shown to deliver smaller drops than the 0.05 gm. required by the specifications. The diameter of the tip which consistently will deliver drops of the required size within the permitted limits of 10 per cent. has been found to be 3.3-3.4 mm.

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SCIENTIFIC AND TECHNICAL ABSTRACTS

Compiled by Linwood F. Tice, M. Sc.

The Preparation of Eye-Drops. J. Büchi and E. Baeschlin. *Pharm. Acta Helv. 11,* 103 (1936).

The Swiss Pharmacopœia in a paragraph pertaining to Collyria makes the following statement: "When eye-drops with alkaloidal salts and borax are prescribed, in case the alkaloidal base is precipitated, the proper amount of boric acid should be dissolved in the liquid, which will prevent the formation of turbidity or a precipitate."

In some cases not only is the alkaloidal base precipitated by the alkalinity, but there is also the possibility of the formation of colored decomposition products, *e. g.,* with physostigmine salicylate.

There is however another point of importance concerning the reaction of eye-drops other than that of chemical incompatibility. Experience has shown that the hydrogen ion concentration of eye-drops should be as near as possible that of the normal tear. The extremely severe pain which is experienced when eye-drops of 1% zinc sulfate (pH about 6.1) are employed or 1% optochin hydrochloride (pH about 6.2, even though such solutions are hypotonic, is due undoubtedly to their acid reaction. The pH of the normal tear fluid has been found to be between 7.15 and 7.35.

The author conducted a series of investigations to determine under what conditions of concentration, pH, etc., various alkaloidal bases would be precipitated from solutions of their salts. Alkaloidal solutions commonly employed in eye-drops were prepared, similar solutions containing various amounts of borax made, the pH measured and the stability of each determined over a period of several weeks. A table is presented in the original paper showing the results of this study.

When boric acid is to be added to an eye-drop formula containing an alkaloidal salt and borax, there is at present no formula which may be used which will provide the proper ratio of the two in order to guarantee stability of the preparation and a pH approximately that of the tear fluid. In order to fill this need the authors prepared a series of boric acid-borax buffer solutions and plotted curves which made it possible to estimate the amount of boric acid which should be added to

various concentrations of borax in order to obtain a pH falling in the region pH 7.15 to 7.35.

The results were as follows:

0.5% borax solution required 1.70-2.00% boric acid
1.0% borax solution required 2.30-2.65% boric acid
2.0% borax solution required 3.05-3.50% boric acid
5.0% borax solution required 4.85-5.50% boric acid

The next step was the preparation of solutions of the various alkaloids in these buffer solutions to ascertain whether such solutions would be stable and whether their pH would be appreciably altered. The assumption that the buffer capacity of the borax-boric acid solution would be sufficient was true for atropine sulfate, homatropine hydrobromide, physostigmine salicylate and scopolamine hydrobromide. For cocaine hydrochloride and pilocarpine hydrochloride smaller amounts of boric acid are necessary to provide this pH.

In regard to the stability, etc., a chart is prepared tabulating the results. Special mention is given the following exceptional results:

Cocaine hydrochloride—The reaction of a 5% solution made up in the various buffer solutions containing 0.5, 1, and 2% borax, was somewhat more acid than expected. When less boric acid was used so as to provide a pH of 7.15-7.35 cocaine base precipitated. Thus the optimum pH from the standpoint of pain is not compatible with stability.

Physostigmine salicylate—The desired pH of 7.15-7.35 is not compatible with this alkaloid inasmuch as a red color develops in a very short time. A quite acid reaction is necessary for the stability of this substance. Thus

0.5% phyostigmine salicylate + 0.5% borax
plus 3% boric acid (pH 6.55) → red color
plus 4% boric acid (pH 6.16) → red color
plus 5% boric acid (pH 5.76) stable

Pilocarpine hydrochloride—The pilocarpine solutions prepared were so acid that an addition of 0.5% of borax did not require the addition of any boric acid. Even 1% borax with 2 and 3% solutions of pilocarpine hydrochloride were compatible.

Cocaine hydrochloride with pilocarpine hydrochloride—The relatively acid reaction of the pilocarpine hydrochloride is evidenced in this combination. With 0.5, 1 and 2% borax smaller amounts of

boric acid are necessary. When this combination is prepared it is important that the following order be followed in dissolving the ingredients:

Boric acid
Pilocarpine hydrochloride
Cocaine hydrochloride
Borax

If the cocaine hydrochloride is dissolved in the buffer solution of borax and boric acid first, it is precipitated as free base which will redissolve, but very slowly.

Physostigmine salicylate with pilocarpine hydrochloride—With this combination a pH at least as low as 5.8 must be obtained in order to prevent the red color due to decomposition of the physostigmine at higher pH levels.

In general it may be said that in the preparation of eye-drops of alkaloidal salts with borax-boric acid additions, the alkaloidal salt should be dissolved in the boric acid solution or with the exception of cocaine salts in the borax-boric acid solution. If one adds, for example, the alkaloidal salt to the borax solution or vice versa the alkaloidal base will be precipitated and it will be dissolved with great difficulty upon the addition of the boric acid.

Use of the Terms "Sterile", "Sterilize" and "Sterilization." Report of the Council on Pharmacy and Chemistry. *J. A. M. A. 107,* 38 (1936). In reply to an official communication from the Food and Drug Administration of the U. S. Department of Agriculture, the Council expressed the following advisory opinion as to the terms "sterile", "sterilize" and "sterilization", especially when it appears on the labels of agents used "for sterilization of the skin".

The Council on Pharmacy and Chemistry has formally gone on record as disapproving of the use of the terms "sterile", "sterilize" and "sterilization" in a bacteriologic sense other than in their correct scientific significance; *i. e.,* meaning the absence or destruction of all micro-organisms. These terms are not relative and to permit their use in a relative sense not only is incorrect but opens the way to abuse and misunderstanding. It is questionable that there are any chemical agents tolerated by the skin which will produce sterility, although there are some which will reduce the bacterial flora of the skin to such

an extent that they may be properly described as disinfecting agents. For such agents there is no objection to the use of the terms "disinfecting", "bactericidal" and "bacteriostatic".

Reference was also made to the decision of the Committee on Foods, which is as follows: The terms "sterile", "sterilized" and "sterilization" shall be used in food advertising in their correct scientific significance only. Foods processed to be free of pathogenic organisms or to keep sound and wholesome are not necessarily sterile, *i. e.*, free from viable micro-organisms.

Eutectic Mixtures. J. Meijer. *Pharm. Weekbl. 72,* 922 (1935), through *Quart. J. Pharm. and Pharmacol. 9,* 130 (1936). In considering the melting point of mixtures of two components, two temperatures may be taken, either that at which melting commences (softening point) or that at which it is complete. The curve of the latter shows a minimum temperature at the eutectic point. On the other hand, the curve of the softening point runs horizontal for most of its course, at a temperature corresponding to the melting point of the eutectic mixture, but rises rapidly at the extremities. When a compound is formed by the two substances a melting point maximum is shown between two eutectics but if the compound decomposes before its melting point is attained only a kink in the curve is observed. In such cases it is also possible to obtain a well-defined curve for the compound by determining instead the curve of the softening point.

A study of the molecular compounds formed by phenazone with other compounds failed to reveal any general laws by which their formation could be detected. Hydrocarbons and alcohols do not combine with it, and aldehydes and ketones combine only if they are halogenated. Many phenols form molecular compounds with phenazone as do aromatic acids and acids containing a halogen or hydroxyl group near the carboxyl group.

A list of low melting eutectic mixtures is given which may be formed in mixtures of powders. Camphor may liquefy with salol, naphthalene, betanaphthol, resorcinol or ethylurethane; salol with monobromated camphor, betanapthol, thymol, guaiacol, napthalene, phenazone, urethane, menthol, chloral hydrate, methylacetenilid, sulphonal, phenacetin or terpin hydrate; phenol with betanaphthol, naphthalene or acetanilid; acetanilid with resorcinol, thymol, chloral hydrate, or methol; thymol with phenacetin or sulphonal; phenazone with methyl-acetanilid, menthol or betanaphthol.

BOOK REVIEW

VITAMINES AND HORMONES.

Dr. Hellmut Bredereck, on the staff of the University of Leipzig, just prepared a booklet (in German), Part 1 on the Results of Vitamine and Hormone research 101, p. Mark 6.00, published by Hirzel, Leipzig.

The monograph (No. 15 of the series on chemistry and technics of our time) is a brief survey of the major results of research in this field. It aims to serve as an introduction into the literature records mainly of chemical, but also of medical and physiological facts. The survey should thus be valuable as a general orientation to workers, including pharmacists, in the Food and Health Sciences.

We find a discussion of the various vitamine deficiency diseases, of the occurrence of vitamines, including the chemically unknown H. & K., of diagnostic tests; composition and synthesis. Similar data are given for the active ingredients of glandular secretion, the hormones.

While the survey presents but a very brief consideration of the wealth of data now available, nevertheless the chapter on the now all-important "sex-hormones" embraces one-fourth of the entire text. Questionable vitamines as Vitamine F are not mentioned, neither are anti-hormones, nor spectroscopic methods of detecting the presence and amount of certain vitamines. A selected list of 341 references to the literature of vitamines and hormones is appended, mainly as a guide in the further study of these indispensable substances.

The valuable survey is a creditable addition to biochemical literature. The second part, now in print, dealing with the technical preparation of vitamines and hormones will, we trust, be an equally worthy contribution.

ARNO VIEHOEVER.